

Serial No. 09/888,824

AMENDMENT TO THE CLAIMS

1- 69. (Cancelled)

70. (Currently amended) A cell-free or cell lysate-free An in vitro screening method for identifying a compound or extract for manufacturing a topical composition for inhibiting lipoprotein lipase (LPL) thereby limiting uptake of fatty acids by adipocytes, wherein said method comprises the steps of:

a) preparing a substrate, wherein the substrate comprises at least one triacylglycerol;

b) placing the substrate in contact with at least

i.) said compound or extract,

ii.) a lipoprotein lipase,

iii.) a cofactor of lipoprotein lipase,

iv.) a fatty acid-acceptor substance or a fatty acid-sequestering substance which avoids or limits the blockage of the enzymatic activity of the lipoprotein lipase for a period of time sufficient for releasing at least in part non-esterified fatty acid from the triacylglycerol: and

c) upon completion of step b), determining the capacity of inhibition of the release from the substrate of the non-esterified fatty acid resulting from the activity of the lipoprotein lipase under the action of said compound or extract, by the monitoring of the release of the non-esterified fatty acid using an enzymatic technique on the reaction medium after completion of step b), ~~under the action of said compound or extract~~ and in case of identified inhibition of LPL activity by said compound or extract, selecting said compound or extract as a compound or extract for manufacturing a the topical composition ~~for limiting the storage of triglycerides.~~

Serial No. 09/888,824

71. (Cancelled)

72. (Previously presented) The method according to claim 70, which comprises a further step of:

d) comparing said determined capacity of inhibition to a control, wherein the control is the capacity of inhibition of LPL activity obtained in the absence of the said compound or extract tested.

73. (Currently amended) The method according to claim 70, which ~~comprises a further step of~~ further comprises the steps of:

~~a) preparing a substrate, wherein the substrate comprises at least one triacylglycerol;~~

~~bd)~~ placing the substrate in contact with at least

i.) a compound known to be an inhibitor ~~known to be active in the field of lipolysis of LPL activity,~~

ii.) a lipoprotein lipase,

iii.) a cofactor of lipoprotein lipase,

iv.) a fatty-acceptor substance or a fatty acid-sequestering substance which avoids or limits the blockage of the enzymatic activity of the lipoprotein lipase for a period of time sufficient for releasing, at least in part, fatty acid from the triacylglycerol; and

ee) upon completion of step ~~bd)~~, determining the capacity of inhibition of the release from the substrate of the fatty acid resulting from the activity of the lipoprotein lipase, under the action of the compound known to be an inhibitor ~~known to be active in the field of lipolysis of LPL activity.~~

74. (Previously presented) The method according to claim 73, wherein the known inhibitor is selected from the group consisting of protamine sulfate, protamine, and sodium pyrophosphate.

Serial No. 09/888,824

75. **(Previously presented)** The method according to claim 74, wherein the cofactor of lipoprotein lipase is of human origin.

76. **(Previously presented)** The method according to claim 70, wherein the fatty acid-acceptor substance or fatty acid-sequestering substance comprises bovine or human albumin.

77. **(Previously presented)** The method according to claim 70, wherein the lipoprotein lipase is obtained from bovine milk or bacteria.

78. **(Previously presented)** The method according to claim 70, wherein the triacylglycerol comprises an acyl part which is obtained from a long chain fatty acid comprising 12 to 30 carbon atoms.

79 -81. **(Cancelled)**

82. **(Previously presented)** The method according to claim 70, wherein the triacylglycerol comprises triolein.

83. **(Previously presented)** The method according to claim 70, wherein said step b) of placing the substrate in contact comprises:

- a) incubating the lipoprotein lipase for a determined period of time in the presence of said compound or extract.
- b) incubating the substrate in the presence of the lipoprotein lipase cofactor;  
and
- c) incubating the mixture of the substrate/lipoprotein lipase cofactor in the presence of the lipoprotein lipase and said compound or extract.

84. **(Previously presented)** The method of claim 70, wherein the lipoprotein lipase cofactor comprises apolipoprotein C-II.

85. **(Currently amended)** The method of claim ~~74~~ 70, wherein the enzymatic technique is ~~observed~~ observation by colormetry for obtaining an optical density value at

Serial No. 09/888,824

a wavelength determined by the particular enzymatic technique utilized, and wherein comparing said determined capacity of inhibition to a control comprises comparing the optical density value obtained at the wavelength.

86. **(Currently amended)** The method of claim 70, wherein the enzymatic technique is ~~observed~~ observation by colorimetry for obtaining an optical density value at 550nm and inhibition is determined by the optical density value at 550nm which expresses a decrease in the non-esterified fatty acids released in the reaction medium, which is compared with the optical density value at 550nm with the control, and the activity of said compound or extract tested is determined by the observation of the inhibition effected by said substance tested with respect to the control.

87 - 90. **(Cancelled)**

91. **(Previously presented)** The method of claim 70, wherein said extract is selected from the group consisting of an aqueous extract of liana *Uncaria tomentosa*, an alcoholic extract of liana *Uncaria tomentosa*, an aqueous alcoholic extract of liana *Uncaria tomentosa*, an aqueous glycolic extract of liana *Uncaria tomentosa*, and a glycolic extract of liana *Uncaria tomentosa*.

92. - 95. **(Cancelled)**

96. **(Currently amended)** The screening method of claim ~~104~~ 99, wherein said triacylglycerol comprises an acyl part comprising 12 to 30 carbon atoms.

97. **(Cancelled)**

98. **(Currently amended)** The screening method of claims ~~108~~ 100, wherein said triacylglycerol comprises an acyl part comprising 12 to 30 carbon atoms.

99. **(New)** A cell-free or cell lysate free in vitro screening method for identifying a compound or extract for reducing storage of triglycerides in adipocytes, wherein said method comprises the steps of:

Serial No. 09/888,824

a) preparing a substrate, wherein the substrate comprises at least one triacylglycerol;

b) placing the substrate in contact with at least

i.) said compound or extract,

ii.) a lipoprotein lipase,

iii.) a cofactor of lipoprotein lipase,

iv.) a fatty acid-acceptor substance or a fatty acid-sequestering substance which avoids or limits the blockage of the enzymatic activity of the lipoprotein lipase for a period of time sufficient for releasing at least in part non-esterified fatty acid from the triacylglycerol; and

c) upon completion of step b), determining the capacity of inhibition of the release from the substrate of the non-esterified fatty acid resulting from the activity of the lipoprotein lipase under the action of said compound or extract, by the monitoring of the release of the non-esterified fatty acid using an enzymatic technique on the reaction medium after completion of step b), and in case of identified inhibition of LPL activity by said compound or extract, selecting said compound or extract as a compound or extract for reducing storage of triglycerides in adipocytes.

100. **(New)** A cell-free or cell lysate free in vitro screening method for identifying a compound or extract for increasing blood microcirculation, wherein said method comprises the steps of:

a) preparing a substrate, wherein the substrate comprises at least one triacylglycerol;

b) placing the substrate in contact with at least

i.) said compound or extract,

ii.) a lipoprotein lipase,

iii.) a cofactor of lipoprotein lipase,

Serial No. 09/888,824

iv.) a fatty acid-acceptor substance or a fatty acid-sequestering substance which avoids or limits the blockage of the enzymatic activity of the lipoprotein lipase for a period of time sufficient for releasing at least in part non-esterified fatty acid from the triacylglycerol; and

c) upon completion of step b), determining the capacity of inhibition of the release from the substrate of the non-esterified fatty acid resulting from the activity of the lipoprotein lipase under the action of said compound or extract, by the monitoring of the release of the non-esterified fatty acid using an enzymatic technique on the reaction medium after completion of step b), and in case of identified inhibition of LPL activity by said compound or extract, selecting said compound or extract as a compound or extract for increasing blood microcirculation.

101. **(New)** A topical composition comprising a compound or extract selected for inhibition of lipoprotein lipase (LPL) activity, wherein the compound or extract is selected by a method comprising the steps of:

a) preparing a substrate, wherein the substrate comprises at least one triacylglycerol;

b) placing the substrate in contact with at least

i.) said compound or extract,

ii.) a lipoprotein lipase,

iii.) a cofactor of lipoprotein lipase,

iv.) a fatty acid-acceptor substance or a fatty acid-sequestering substance which avoids or limits the blockage of the enzymatic activity of the lipoprotein lipase for a period of time sufficient for releasing at least in part non-esterified fatty acid from the triacylglycerol; and

c) upon completion of step b), determining the capacity of inhibition of the release from the substrate of the non-esterified fatty acid resulting from the activity of

Serial No. 09/888,824

the lipoprotein lipase under the action of said compound or extract, by the monitoring of the release of the non-esterified fatty acid using an enzymatic technique on the reaction medium after completion of step b), and in case of identified inhibition of LPL activity by said compound or extract, selecting said compound or extract as a compound or extract for manufacturing the topical composition.

102. **(New)** The topical composition of claim 101 wherein the topical composition may be utilized for decreasing fatty deposits or decreasing the rate of fatty deposits *in vivo*; for improving the appearance of human skin, or for diminishing the "orange peel" appearance of skin.